

CLAIMS:

1. A method of inducing or promoting dopaminergic neuronal development by enhancing proliferation, self-renewal,
5 dopaminergic induction, survival, differentiation and/or maturation in a neural stem, progenitor or precursor cell, or other stem or neural cell, the method comprising:
expressing a nuclear receptor of the *Nurr1* subfamily above basal levels within the cell, and
10 treating the cell with a Wnt ligand, thereby producing or enhancing proliferation, self-renewal, survival and/or dopaminergic induction, differentiation, survival or acquisition of a neuronal dopaminergic phenotype.
- 15 2. A method according to claim 1 wherein the nuclear receptor is *Nurr1*.
3. A method according to claim 1 wherein the nuclear receptor is *Nor1* or *NGFI-B*.
- 20 4. A method according to any one of the preceding claims comprising expressing *Nurr1* above basal levels by transforming a cell with *Nurr1* DNA or introducing into the cell *Nurr1* RNA.
- 25 5. A method according to any one of claims 1 to 3 comprising expressing *Nurr1* above basal levels by introducing *Nurr1* protein into the cell.
- 30 6. A method according to any one of claims 1 to 3 comprising expressing *Nurr1* above basal levels by preserving *Nurr1* protein in the cell.

7. A method according to any one of claims 1 to 6 wherein the Wnt ligand is a Wnt1 ligand.

8. A method according to any one of claims 1 to 7 wherein the Wnt ligand is a Wnt5a ligand.

9. A method according to any one of the preceding claims wherein the Wnt ligand is Wnt3a.

10. A method according to any one of the preceding claims, wherein the Wnt ligand is Wnt-2.

11. A method according to any one of the preceding claims, wherein the Wnt ligand is Wnt-4.

12. A method according to any one of the preceding claims, wherein the Wnt ligand is Wnt-7a.

13. A method according to any one of the preceding claims, wherein the Wnt ligand is Wnt-7b.

14. A method according to any one of the preceding claims wherein said neural stem, progenitor or precursor cell or other stem cell or neuronal cell is treated with Wnt ligands other than Wnt-1 or Wnt-5a or an additional Wnt ligand.

15. A method according to any one of the preceding claims wherein the neural stem, progenitor or precursor cell or other stem or neural cell is mitotic and/or capable of self-renewal when it is treated with the Wnt ligand.

16. A method according to any one of the preceding claims wherein said neural stem, progenitor or precursor cell or other stem or neural cell is additionally contacted with a member of the FGF family of growth factors.

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17. A method according to any one of the preceding claims wherein said neural stem, progenitor or precursor cell or other stem or neural cell is contacted with a retinoid or retinoid derivative, an activator of the retinoid X receptor (RXR), a repressor of the retinoid acid receptor (RAR), 9-cis retinal, DHA, SR11237, or LG849.

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18. A method according to any one of the preceding claims wherein the neural stem, progenitor or precursor cell or other stem or neural cell is treated with bFGF and/or EGF and/or FGF-8 and/or LIF and/or Shh prior to or simultaneously with treating the cell with a Wnt ligand.

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19. A method according to any one of the preceding claims wherein the neural stem, progenitor or precursor cell or other stem or neural cell is grown in the presence of antioxidants, ascorbic acid, low oxygen tension or a hypoxia-induced factor..

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20. A method according to any one of the preceding claims wherein the neural stem, progenitor or precursor cell or other stem or neural cell grows and/or differentiates in the presence of ventral mesencephalic astrocytes or early glial cells.

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21. A method according to any one of the claims 1 to 20 wherein the Wnt ligand is added to an *in vitro* culture containing the cell.

22. A method according to claim 21 wherein Wnt ligand is produced by expression from a cell co-cultured with the neural stem, progenitor or precursor cell, or other stem or neural cell, which co-cultured cell is a cell other than a type 1 astrocyte or early glial cell or is a host cell transformed with nucleic acid encoding the Wnt ligand or a cell containing introduced Wnt protein.

23. A method according to claim 22 wherein the co-cultured cell other than a type 1 astrocyte or early glial cell or host cell is another stem, neural stem, progenitor, precursor or neural cell.

24. A method according to claim 21 wherein the neural stem, progenitor or precursor cell, or other stem or neural cell, is engineered to express the Wnt ligand from encoding nucleic acid.

25. A method according to claim 21, wherein Wnt ligand protein is introduced into the cell.

26. A method according to any one of claims 1 to 25 comprising further co-culturing the neural stem, progenitor or precursor cell, or other stem or neural cell, with an early glial cell, or a Type 1 astrocyte optionally of the ventral mesencephalon.

27. A method according to claim 26 wherein the Type 1 astrocyte is immortalized or is of an astrocyte cell line of a region other than the ventral mesencephalon.

28. A method according to any one of the preceding claims, comprising additionally contacting the neural stem, progenitor or precursor cell, or other stem or neural cell with a negative selection agent that selects against non-dopaminergic neurons.

29. A method according to any one of the preceding claims further comprising formulating a neuron into a composition comprising one or more additional components.

30. A method according to claim 29 wherein the composition comprises a pharmaceutically acceptable excipient.

31. A method according to claim 30 further comprising administering the composition to an individual.

32. A method according to claim 31 wherein the neuron is implanted into the brain of the individual.

33. A method according to any one of claims 1 to 20 wherein the cell is treated in an individual *in situ* to increase *Nurrl* expression.

34. A method according to any one of claims 1 to 20 wherein the neural stem, progenitor or precursor cell or other stem or neural cell is treated in an individual *in situ* with a Wnt ligand.

35. A method according to claim 34, wherein nucleic acid encoding the Wnt ligand is introduced into the cell.

36. A method according to claim 34, wherein Wnt ligand protein is introduced into the cell.

37. A method according to claim 34 wherein the cell is treated in an individual *in situ* to increase *Nurr1* expression above basal levels.

5 38. A method according to any one of claims 33 to 37 wherein the neural stem, progenitor or precursor cell or other stem or neural cell is endogenous to the individual.

10 39. A method according to any one of claims 33 to 37 wherein the neural stem, progenitor or precursor cell or other stem or neural cell is exogenously supplied by grafting into the individual.

15 40. A method according to any one of claims 31 to 39 wherein the individual has Parkinson's disease, a parkinsonian syndrome, neuronal loss or a neurodegenerative disease.

20 41. A method according to any one of claims 1 to 30 further comprising use of a neuron produced in accordance with the method in the manufacture of a medicament for treatment of an individual.

25 42. A method according to claim 41 wherein the medicament is for implantation into the brain of the individual.

43. A method according to claim 42 wherein the individual has Parkinson's disease, a parkinsonian syndrome, neuronal loss or a neurodegenerative disease.

30 44. A dopaminergic neuron produced in accordance with any one of claims 1 to 28.

45. Use of a dopaminergic neuron according to claim 44 in a method of screening for an agent for use in treatment of a neurodegenerative disease.

5 46. A method according to any one of claims 1 to 28 further comprising:

(i) treating a dopaminergic neuron with a toxin for said dopaminergic neuron;

(ii) separating the dopaminergic neuron from the toxin;

10 (iii) bringing the treated dopaminergic neuron into contact with a test agent or test agents;

(iv) determining the ability of the dopaminergic neuron to recover from the toxin;

(v) comparing said ability of the dopaminergic neuron to
15 recover from the toxin with the ability of a dopaminergic neuron to recover from the toxin in the absence of contact with the test agent or test agents.

20 47. A method according to any one of claims 1 to 28 further comprising:

(i) treating a dopaminergic neuron with a toxin for the dopaminergic neuron in the presence of a test agent or test agents;

25 (ii) determining the ability of the dopaminergic neuron to tolerate the toxin;

(iii) comparing said ability of the dopaminergic neuron to tolerate the toxin with the ability of a dopaminergic neuron to tolerate the toxin in the absence of contact with the test agent or test agents.

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48. A method according to claim 46 or claim 47 further comprising formulating an agent which improves ability of a dopaminergic neuron to recover from or tolerate a said toxin into a composition comprising one or more additional
5 components.

49. A method according to claim 48 wherein said composition comprises a pharmaceutically acceptable excipient.

10 50. A method according to claim 49 further comprising administering said composition to an individual.

51. A method according to claim 50 wherein the individual has Parkinson's disease, a parkinsonian syndrome, neuronal loss or
15 a neurodegenerative disease.

52. A method of obtaining a factor or factors which, either alone or in combination, enhance proliferation, self-renewal, survival and/or dopaminergic development, induction,
20 differentiation, or maturation in a neural stem, progenitor or precursor cell, or other stem or neural cell expressing *Nurr1* above basal levels, the method comprising:

(a) treating a neural stem progenitor or precursor cell, or other stem or neural cell expressing *Nurr1* above basal
25 levels with a Wnt ligand in the presence and absence of one or more test substances; and

(b) determining proliferation, self-renewal, survival and/or dopaminergic development, induction, differentiation, or maturation of the cell and comparing the extent of the
30 proliferation, self-renewal, survival and/or dopaminergic development, induction, differentiation or maturation in the presence and absence of the test substance or substances, whereby said factor or factors is obtained.

53. A method according to claim 52 wherein the cell is treated with the Wnt ligand by addition of the Wnt ligand to *in vitro* culture containing the cell.

5 54. A method according to claim 52, wherein the cell is treated with the Wnt ligand by introduction of nucleic acid encoding the Wnt ligand into the cell.

10 55. A method according to claim 52, wherein the cell is treated with the Wnt ligand by introduction of Wnt ligand protein into the cell.

15 56. A method according to claim 53 wherein the neural stem, progenitor or precursor cell, or other stem or neural cell is treated with the Wnt ligand by co-culturing with a cell which is a cell other than a type 1 astrocyte or early glial cell or is a host cell transformed with nucleic acid encoding the Wnt ligand or a cell containing introduced Wnt protein.

20 57. A method according to any one of claims 52 to 56 further comprising co-culturing the neural stem, progenitor or precursor cell, or other stem or neural cell with an early glial cell or a Type 1 astrocyte optionally of the ventral mesencephalon.

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58. A method according to any one of claims 52 to 57 wherein a factor or factors able to enhance proliferation, self-renewal, survival and/or dopaminergic development, induction, differentiation or maturation in a neural stem, progenitor or precursor cell, or other stem or neural cell expressing *Nurr1* above basal levels is or are provided in isolated and/or purified form.

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59. A method according to any one of claims 56 to 58 wherein a factor or factors able to enhance proliferation, self-renewal, survival and/or dopaminergic development, induction, differentiation or maturation in a neural stem, progenitor or precursor cell, or other stem or neural cell expressing *Nurr1* above basal levels is or are formulated into a composition comprising one or more additional components.

60. A method according to claim 59 wherein the composition comprises a neural stem, progenitor or precursor cell, or other stem or neural cell expressing *Nurr1* above basal levels.

61. A method according to claim 60 wherein the composition comprises Wnt ligand.

62. A method according to claim 61 wherein the Wnt ligand is a Wnt1 ligand.

63. A method according to claim 61 wherein the Wnt ligand is a Wnt5a ligand.

64. A method according to any one of claims 59 to 63 wherein the composition comprises a pharmaceutically acceptable excipient.

65. A method according to claim 64 further comprising administering the composition to an individual.

66. A method according to claim 65 wherein the composition is implanted into the brain of the individual.

67. A method according to claim 66 wherein the individual has Parkinson's disease, a parkinsonian syndrome, neuronal loss or a neurodegenerative disease.